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## Single-case experimental designs for behavioral neuroscience

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Single-case experimental designs (SCEDs) are commonly used in behavior analytic research but rarely used in behavioral neuroscience research. The recent development of technologies that allow control of the timing of neurobiological events such as gene expression and neuronal firing enable the fruitful application of SCEDs for the study of brain–behavior relations. There are at least 3 benefits expected from applying SCEDs to study how neurobiological events affect behavior. First, SCEDs entail direct within- and across-subject assessments of reliability, likely increasing the probability of replication across studies and encouraging a search for the causes of replication failure when they occur. Second, SCEDs focus on behavior in individual organisms producing a body of knowledge that applies to individuals rather than population parameters. Finally, SCEDs require fewer animals, decreasing costs and effort and addressing the ethical obligation to reduce the number of animals used for research. Examples are provided using hypothetical data generated based on published research. Collaborations between behavior analysts and behavioral neuroscientists will bring the world within the skin under direct experimental control and broaden our understanding of the determinants of behavior.

*Key words:* single-case experimental designs, behavioral neuroscience, optogenetic, DREADD, gene expression

In behavioral research, single-case experimental designs (SCEDs) refer to a family of research designs in which the focus of analysis is the individual animal or human participant (Iversen, 2013; Perone & Hursh, 2013). SCEDs involve repeated measurements of behavior before, during, and after an experimental manipulation to evaluate the manipulation's behavioral effect. SCEDs have long been used in behavioral research (Iversen, 2013) and were utilized by B. F. Skinner for the study of operant behavior (Skinner, 1938). SCEDs currently are used in basic experimental and applied research in the field of behavior analysis. The value of SCEDs is increasingly recognized in areas outside of behavior analysis such as medicine (Gabler et al., 2011), rehabilitation (Krasny-Pacini & Evans, 2018; Lobo et al., 2017), clinical psychology (Sexton-Radek, 2014), health psychology (Dallery

et al., 2013; Kwasnicka et al., 2019), and psychiatry (Marwick et al., 2018).

Although heavily used in behavior analytic research, SCEDs are rarely used in behavioral neuroscience research. For example, a targeted review of articles published in seven neuroscience journals between 7/31/2019 and 8/31/2020 was conducted that focused on studies that used both an operant procedure as well as neuroscience techniques suitable for use of within-subject experimental designs (e.g., optogenetics and chemogenetics; see supplemental material for search details). For studies involving optogenetics, the search found that although 83% (20/39) of the articles involved a within-subject manipulation of optogenetic stimulation or inhibition, only 35% (7/20) of those articles reported repeated observations that would allow an assessment of reliability of the effect of the manipulation. Further, of those articles that did report repeated observations, only 43% (3/7) reported at least three data points within an experimental condition, which is considered the minimum required (Kratochwill et al., 2010). Finally, only 5% (1/20) of the studies that involved a within-subject manipulation of optogenetic stimulation or inhibition reported results from repeated conditions or reversals (defined as implementation of an experimental manipulation for a period of time or for a specific response

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followed by a change [e.g., removal of the manipulation or application of the manipulation to a different response] for a period of time followed by a return to the original condition). Similarly, of the studies identified that reported results of chemogenetic manipulations on operant behavior, only 50% (4/8) used a within-subject chemogenetic manipulation and of those that did, 0% reported repeated observation or repeated condition data. Thus, it seems clear that SCEDs are underutilized in neuroscience research and when studies do utilize within-subject manipulations, the methodological details do not meet minimal standards for establishing confidence in the effect of the independent variable (see Kratochwill et al., 2010). Despite the infrequent use of SCED studies in neuroscience, the relatively recent development of technologies that allow control over the timing of biological events such as gene transcription and neuronal stimulation or inhibition (e.g., optogenetics) enables the use of SCED methods.

The goal of this manuscript is to encourage collaborations between behavior analysts and neuroscientists and to provide some hypothetical examples of how behavioral studies using SCEDs could be conducted to address current topics in behavioral neuroscience. To a large degree, the content of this paper is an amalgamation of arguments made convincingly by others. Other authors have elucidated the importance of rigorous behavioral study as a prerequisite for the investigation of the necessary and sufficient neural substrates of behavior (Krakauer et al., 2017). Other authors have argued convincingly that behavior analysis, with its focus on observable, measurable behavior in individual organisms and rigorous methodology, is particularly compatible with behavioral neuroscience (Donahoe, 2017; Schlinger, 2015). Finally, other authors have raised concerns about the future of basic research in behavior analysis (e.g., Poling, 2010) and have argued that behavior analysis would benefit from collaborations with neuroscience researchers (e.g., Fox, 2018; Kangas, 2014; Marr & Zilio, 2013). Building on those arguments, the focus of this manuscript will be to argue for the benefits of SCEDs for behavioral neuroscience research and to suggest how such studies might be conducted using hypothetical data generated based on published behavioral neuroscience research.

Appreciation of the benefits for behavioral neuroscientists of adopting SCEDs requires an introduction to SCEDs, their logic, and methodological details. For behavior analytic readers, the material will be familiar, but for the non-behavior-analytic reader, the coverage is necessary. For behavior analytic readers, the later neuroscience material will be unfamiliar and thus an introduction to the technologies is provided.

### **Potential Barriers to Use of Single-Case Experimental Designs**

Two potential barriers to the adoption of SCEDs in behavioral neuroscience research should be addressed prior to further discussion (also see Aeschleman, 1991; Dallery & Raiff, 2014 for a discussion of these and other misconceptions). First, one potential barrier to the use of SCEDs in behavioral neuroscience research is a misunderstanding that SCEDs fail to control for confounding variables (i.e., threats to internal validity; Campbell, 1957). Of most concern in single-case research are threats to internal validity that derive from the passage of time or from repeated exposure to the experimental manipulation because the designs involve comparing behavior of an individual across periods of time in which an experimental manipulation is repeatedly administered (Perone & Hursh, 2013). Those threats include history (something else happened), maturation (changes in the organism under study), testing (changes in behavior due to repeated assessment of the behavior), and instrumentation (changes in the measurement apparatus). In contrast to group designs that attempt to control for threats to internal validity via random assignment of subjects to control and experimental groups, SCEDs effectively address these threats by replicating control and experimental conditions (Perone & Hursh, 2013; Sidman, 1960). To the degree that results are demonstrated reliably across replicated conditions, confidence in the effects of the experimental manipulation on the dependent variable increase (i.e., the internal validity of the experiment increases).

A second potential barrier to adoption of SCEDs in behavioral neuroscience research is a misunderstanding that SCEDs do not produce generalizable results (i.e., they fail to

establish external validity) because they involve fewer subjects than group designs and often do not utilize null hypothesis significance testing (NHST). As clearly explained by Branch and Pennypacker (2013), the generality provided by group designs and NHST of group design data applies to inferences about population parameters rather than to the individual organisms that constitute the sample or population. The generality provided by group designs is often mistakenly thought to apply to individuals and is thus used as an argument in favor of group designs over SCEDs.

As described by Branch (2014), there are two ways in which group designs and the typical analysis of group designs via NHST may lead to erroneous conclusions about individuals. First, group means may not be representative of many or even one subject (e.g., a marriage rate of 48% in the population does not apply to any individual in the population—no one is 48% married). This can be an issue when a therapeutic intervention is implemented for an individual patient based on group results (Kravitz et al., 2004; Williams, 2010). Further, when an independent variable is manipulated quantitatively over a range of values for each subject to determine the relation between the independent and dependent variable, it also is the case that averaging across subjects may yield a relation that fails to accurately depict the relation in the individual subjects (see Branch, 2019 for an example from dark adaptation; see Gallistel et al., 2004 for a discussion of the issue related to learning curves).

A second, more subtle way in which group designs may lead to erroneous conclusions about individuals may arise when different groups are used to evaluate the effects of each value of a quantitatively varied independent variable. In such a circumstance, the resulting relation between the independent and dependent variables may not be representative of the relation for the individuals. This is because no individual can experience every level of the independent variable without having experienced one or more levels of the independent variable previously (i.e., experience every level of the independent variable without any history of prior exposure to the independent variable). For example, if prior drug exposure affects subsequent response to a drug, the

dose-effect curve obtained using separate groups of animals for each dose and the dose-effect curves obtained via repeated dosing in individual animals likely would differ (see Branch, 2019; Branch & Pennypacker, 2013; Sidman, 1960 for other examples and further discussion of this issue). For those interested in phenomena at the individual organism level, this is not a complication that can be circumvented by using group designs. Thus, although group designs may avoid some of the issues related to repeated assessments of a variable's effects, the results obtained may not correspond with results that would be obtained with individual subjects.

In summary, SCEDs produce generalizable results in the most direct manner possible, via systematic replication, establishing whether previously observed effects of an experimental manipulation apply across conditions different from those of the original experiment (Sidman, 1960). Importantly, SCED findings systematically replicated across studies establish the external validity of the findings and apply to individuals rather than groups of individuals (Branch & Pennypacker, 2013). That is, the more a finding is replicated at the individual level across studies that vary in terms of types of participants/subjects, settings, and other features, the greater the generality of the finding at the individual participant/subject level.

## Overview of Single-Case Experimental Designs

The use of SCEDs to study phenomena in whole, living organisms can be traced back at least as far as Claude Bernard's work on physiology and subsequently to Ivan Pavlov's work on classical conditioning (Iversen 2013). Thus, although the use of SCEDs in psychology today is primarily limited to the experimental and applied analyses of behavior, their use extends beyond those areas historically.

Research using SCEDs is based on a philosophical position, supported by a wealth of data, that the behavior of individual organisms is orderly and can be the subject of scientific inquiry. It is, of course, the case that individual organisms behave, and those behaviors produce effects on the environment that may subsequently alter the behavior of the organism. It is the individual that burns her hand when she touches a hot stove, not the group. It also

is the case that organisms have nervous systems whose function underlies behavior and that those nervous systems are not shared between organisms. Finally, it also is the individual who must be treated when behavior becomes problematic (e.g., when obsessive-compulsive behavior interferes with an individual's daily functioning). Thus, from a SCED perspective, behavior is a phenomenon that occurs in individual organisms and as a result, can be studied at the individual level.

SCEDs in behavioral research evaluate the impact of an experimental manipulation on a behavioral outcome for an individual organism. Determination of the impact of an experimental manipulation rests on repeated observations of the behavior during control and experimental conditions and comparison of the resulting series of observations from those conditions. SCEDs are particularly well-suited to experimental investigations of the determinants of behavior because behavior is a phenomenon that applies uniquely to individual organisms. Similarly, nervous system functioning is a phenomenon that applies uniquely to individual organisms, and as such SCEDs should be useful for evaluating the impact of neurobiological manipulations such as changes in gene expression and neuronal activity on behavior. It is important to note that the designs described below do not encompass all possible single-case experimental approaches. Designs can be modified and combined to provide confidence in the effects of the experimental manipulation.

## **Types of Designs**

### ***Reversal Designs***

A single-case reversal design involves repeated observations of behavior from each of at least three conditions. The A-B-A reversal design first implements a baseline condition (A) during which the manipulation of interest is not applied, followed by an experimental condition during which the manipulation of interest is applied (B), followed by a return to the baseline condition (A). Importantly, repeated observations of behavior are obtained during each condition and implementation of the experimental condition and the return to the baseline condition are typically not conducted until stability of behavior is achieved in the preceding condition (for a discussion of issues relating to stability of

behavior see Perone and Hursh 2013). Thus, in an ideal scenario, a clear change in behavior during the experimental condition followed by a return of behavior to baseline levels following a return to the baseline condition provides a strong argument for the experimental manipulation being the cause of the behavioral change rather than other variables (threats to internal validity). For example, from a behavioral neuroscience perspective, a SCED could evaluate the impact of a manipulation such as transcription of a gene by comparing repeated behavioral observations during a time period when the gene was being transcribed (B condition) to repeated behavioral observations during time periods prior to (initial A condition) and after (second A condition) the period during which the gene was transcribed.

The A-B-A reversal design can be extended to include replication of the experimental intervention. An A-B-A-B reversal design therefore entails a baseline condition, followed by an experimental manipulation condition, followed by a second baseline condition, followed by a final experimental manipulation condition. As with the A-B-A design, each condition is implemented after behavior reaches stability in the preceding condition. The question is whether behavior changes in an orderly fashion with implementation (observed twice) and removal (observed once) of the experimental condition. Of course, variations of reversal designs are endless and can include any number of replications of the A and B conditions as well as other experimental manipulation conditions (e.g., an A-B-C-A-B-C-A reversal design with a repeated baseline condition labeled A and two repeated experimental conditions labeled B and C). Importantly, the degree to which the pattern of behavior observed during the first baseline condition and changes in behavior observed during the first experimental condition recur during subsequent baseline and experimental conditions, respectively, determines the confidence of the researcher that the experimental manipulation, and not some alternative confounding variable, is responsible for the observed behavioral changes.

### ***Multiple Baseline Designs***

Multiple baseline designs offer a single-case approach to evaluate the behavioral impact of

an experimental manipulation that cannot be reversed (e.g., permanent deletion of a gene). One type of multiple baseline design is the multiple-baseline design across cases in which the experimental manipulation is replicated across individual subjects in a staggered fashion such that each subject is exposed to the baseline condition for differing lengths of time. Once behavior is deemed stable for the first subject, the experimental manipulation is made for that subject. Importantly, the effect of the experimental manipulation on behavior is assessed for the first subject before the manipulation is implemented for the second subject and so on. Maintaining the baseline condition for subsequent subjects while the experimental manipulation is imposed for a previous subject allows the researcher to use the baseline performances of the subsequent subjects as a control for confounding variables. In this respect, a multiple baseline design is unlike the other SCEDs because it involves comparison across subjects to increase confidence in the effect of the manipulation on the behavior of an individual subject. If behavior changes for each subject only after the experimental manipulation and simultaneously does not change for subjects for whom the manipulation has not been implemented, alternative explanations become implausible. For example, a multiple baseline across cases design could be used to evaluate the behavioral impact of a nonreversible neurobiological manipulation such as gene deletion or knockout. Staggering the timing of the gene deletion for different subjects could be used to determine whether behavior changes after and only after the gene is deleted for each subject.

### ***Multielement Design***

A multielement design can be used to evaluate the behavioral effect of one or more experimental manipulations by frequently changing conditions to assess the impact of each condition. The effect of the independent variable on the dependent variable is established for an individual subject when there is a clear differentiation in responding between conditions. For example, application of a multielement design to evaluate a neuroscientific question such as how light stimulation of neurons (optogenetic stimulation)

alters behavior could involve arranging, within experimental sessions, time periods during which no manipulation is implemented, time periods during which neurons are stimulated (i.e., produces neuronal firing) via light exposure, and possibly time periods during which neurons are exposed to light of a wavelength that does not modify neuronal firing (see Optogenetics section below). If behavior differs in a systematic fashion across the different conditions, one's confidence that behavioral differences are due to the differences implemented across conditions is increased. Importantly, the reliability of the differences obtained across conditions addresses the internal validity of conclusions of the independent variable's causal impact.

### ***Factorial Design***

A factorial design is one in which two or more experimental variables are manipulated and the effects of all possible combinations of the imposed levels of the variables examined. A single-case experiment factorial design involves exposing each subject to all combinations of the manipulated variables. Such designs may be particularly useful in behavioral neuroscientific investigations where the impact of a brain manipulation may depend on one or more environmental variables. For example, the behavioral effect of inducing or silencing expression of a gene coding for a neurotransmitter receptor may depend on parameters such as the value of the schedule of reinforcement. Threats to internal validity relating to the passage of time can be addressed by replication of one or more conditions (i.e., combinations of the variables).

### ***Parametric Design***

A parametric design is one in which an experimental manipulation is varied quantitatively over three or typically more values. If behavior varies in an orderly manner with changes in the values of the experimental variable, confidence in the causal impact of the experimental manipulation increases. If conditions are arranged in a strictly ascending or descending sequence (e.g., increasing or decreasing values of reinforcer magnitude or drug dose), threats to internal validity relating to time (e.g., maturation, testing) can be eliminated by replicating selected conditions

(Perone & Hursh, 2013). Alternatively, conditions can be arranged in a random-ordered sequence. Parametric designs can be used in behavioral neuroscience to assess parametrically the effects of a neurobiological manipulation on behavior in individual organisms. For example, a parametric design could be used to assess how optogenetic stimulation or inhibition of neurons affects demand for a reinforcer, assessed across a series of fixed-ratio (FR) schedules of reinforcement.

### General Considerations

One issue that naturally arises in consideration of SCEDs is that of how many observations are needed within an experimental condition and how many intra- and intersubject replications are necessary to produce confidence in the effect of the independent variable. Unfortunately, there are no definitive answers because the answers depend on multiple factors such as the likelihood of the results considering previously established findings, the magnitude of the effect, the variability of the behavior, the time needed for behavior to reach stability, and the goals of the experimenter (see Branch and Pennypacker, 2013, for a discussion). Although guidelines have been proposed (e.g., Kratochwill et al., 2010), ultimately such decisions must be made by the scientist and must be convincing to their peers.

A related issue is how to approach failures to replicate, either within or across subjects. Failures to replicate the effect of an independent variable manipulation may lead one to conclude that previous demonstrations of effect were anomalies and/or due to chance. However, as discussed by Perone (2019), if effects were demonstrated convincingly in other subjects or in previous conditions, such a conclusion would be unjustified. Rather, if a manipulation can be demonstrated to produce a reliable effect for an individual but does not yield the same outcomes for another individual (or in later conditions for the first individual), what is revealed is a lack of understanding and/or control of all relevant variables. As Perone details, investigations that are conducted to address these gaps in understanding can increase our knowledge of the relevant variables (see Clark and Steele 1963 for an example of how differences in the behavioral effects of chlorpromazine between

two rats were resolved by subsequent experimental manipulations).

A third issue for consideration in the use of SCEDs involves independent variable effects that are irreversible or long-lasting. Examples (e.g., math acquisition, language learning, brain lesions) are numerous. The use of irreversible manipulations such as brain lesions in neuroscience may have contributed to the lack of use of SCEDs in neuroscience. Further, irreversible manipulations that are time-sensitive (e.g., early-life manipulations that produce long-lasting permanent changes) are not suitable for investigation with SCEDs. As described above, irreversible manipulations that can be implemented at different timepoints for different subjects (multiple baseline design) can be used to study irreversible effects in individual subjects. Additionally, assessing behavioral changes at the individual subject level when a manipulation produces long-lasting effects is possible. For example, when repeated administration of a drug produces tolerance, a dose-effect curve can be evaluated before, during, and after chronic drug treatment to establish changes in drug effect for each subject (e.g., Marusich & Branch, 2009; Minervini & Branch, 2013). Thus, although irreversible and long-lasting effects represent a challenge for SCEDs, and in certain cases may necessitate group comparisons, the fact that a manipulation produces irreversible or long-lasting effects does not, by itself, render the study of that manipulation out of reach for SCEDs.

Finally, it is important to note that SCED research traditionally has relied on visual analysis of data to ascertain effects of experimental manipulations as opposed to NHST. One reason for this is that NHST focuses on group comparisons whereas SCEDs focus on within- and between-subject replications as the threshold for concluding an effect of an independent variable. The individual subject focus and avoidance of NHST may have shielded SCED research from replication concerns. As documented in a large body of literature, NHST is associated with many issues (Branch, 2014), including a common misunderstanding that *p* values indicate the reliability of a research finding (Cohen, 1994; Falk & Greenbaum, 1995; Haller & Kraus, 2002; Oakes, 1986; Sohn, 1998). This misunderstanding of *p* values has been identified as another factor

contributing to the “replication crisis” in psychology because the misunderstanding discourages conduct of actual replications (Branch, 2019; Perone, 2019). Further, misunderstandings and misuse of  $p$  values in NHST are so pervasive that a recent editorial in an issue of the *Journal of the American Statistical Association* called for an end to use of the phrase “statistical significance” and the dichotomization of results into statistically and not statistically significant (Wasserstein et al., 2019). Importantly, statistical methods for the analysis of SCED data that are not simply focused on rejection of the null hypothesis are available (e.g., Parker et al., 2011) and being developed (see Fisher & Lerman, 2014; Young, 2019). Additionally, standard statistical significance approaches can be used to bolster the analysis of data when needed (e.g., when journals require  $p$ -values for publication). Regardless of whether a visual, statistical, or combined analysis is undertaken, by focusing on an assessment of effects at the individual subject level, SCEDs are not subject to the issues inherent with NHST and can deliver what some mistakenly believe NHST provides: a direct assessment of the within- and between-subject reliability of the findings.

### Benefits for the Use of SCEDs

Researchers in behavioral neuroscience could benefit from adoption of SCEDs. First, SCEDs entail direct within- and across-subject assessments of reliability, likely increasing the probability of replication across studies (Branch, 2019) and encouraging a search for the causes of replication failure when failures occur (Perone, 2019). SCEDs assess reliability of results within an experiment by conducting repeated measurements within conditions and by repeating conditions within and across subjects. The importance of replication has long been recognized (e.g., Cohen, 1994; Ioannidis, 2013a). In fact, a lack of replication attempts has been identified as one factor contributing to the “replication crisis” in psychology and other areas of science (Ioannidis, 2013a, 2013b; Lilienfeld, 2017; cf. Open Science Collaboration, 2015; Pashler & Harris, 2012). As noted by Branch (2019), it is hard to imagine how experiments that involve within- and between-subject assessments of reliability could

generate findings with lower likelihood of replication than is currently estimated in psychology.

A second benefit of SCEDs for behavioral neuroscientists is that use of SCEDs generates a body of knowledge regarding processes that apply to the individual organism rather than a population parameter (Branch, 2014; Branch & Pennypacker, 2013; Sidman, 1960). As described above, behavior is properly construed as a phenomenon that occurs at the individual organism level and thus a science of behavior should generate knowledge that applies at the individual organism level. This is contrasted with the results obtained from group designs, which may apply to the population from which the samples used were drawn, but do not typically permit inferences to be made about individuals comprising the samples.

Finally, SCEDs require fewer animals which decreases costs and effort and addresses the ethical obligation to reduce the number of nonhuman animals used for research. These benefits derive from the fact that, by using each animal as its own control, SCEDs can reduce the number of animals required to convincingly demonstrate an experimental effect. As a practical matter, a reduction in the number of animals translates to a reduction in research costs and labor. A reduction in the number of animals may be of particular benefit in behavioral neuroscience for two reasons. First, many of the techniques are procedurally involved (e.g., stereotaxic surgery). Second, by providing an avenue to reduce the number of animals used, a SCED approach provides a viable alternative to calls to greatly increase sample sizes (e.g., sample sizes in the hundreds of animals) to address low power in group design studies (Button et al., 2013). Although the focus of this paper is on research with nonhuman animals, it may be of interest that others have called for greatly increased sample sizes to address low power in group design studies with humans (Turner et al., 2018). Thus, the arguments made here suggest equivalent benefits with respect to human research. As an ethical matter, a reduction in the number of animals addresses the ethical objective to use the minimum number of nonhuman animals necessary to obtain information, one of the three “Rs” (reduce, refine, replace) of ethical research with nonhuman animals (Russell & Burch, 1959).



### Technologies for Behavioral Neuroscience

Technologies are available that enable control over neurobiological processes such as gene expression in neurons and the activity of neurons in brain-region and neuron type-specific, manners. Critically, available technologies enable control over the timing of such manipulations, and it is therefore possible to investigate brain-behavior relations using SCEDs. A brief discussion of each of these technologies follows, along with examples from the literature of the use of these technologies to address behavioral questions. Each example from the literature is considered from the standpoint of how a SCED could be used to evaluate the effect of the employed manipulations on behavior.

In the examples detailed below, the investigations of the behavioral effects of neurobiological variables can be viewed as bridging the fields of neuroscience and behavior analysis by evaluating the neural substrates and processes that mediate and/or modulate the behavioral functions of environmental stimuli. Another approach, and possibly one of more interest to behavior analysts, is to investigate neurobiological variables as stimuli with reinforcing, punishing, and discriminative functions or as motivating operations that alter the reinforcing or punishing effectiveness of other stimuli (cf. Ortu & Vaidya, 2017; Thompson, 2007). Although some examples of this approach exist, they are far fewer than approaches to identifying substrates that mediate and/or modulate behavioral phenomena. Suggestions for future research on the role of neurobiological variables as variables with stimulus functions or motivating effects are suggested in the conclusion of this paper.

### Technologies for Controlling Gene Expression

Two approaches to control gene expression are to (1) control gene expression via delivery of an exogenous agent to animals genetically engineered such that transcription of a target gene depends on the presence or absence of the exogenous agent (Das et al., 2016) and (2) deliver genetic material into an organism via a viral vector such as adeno-associated virus (AAV; Naso et al., 2017). Often technologies are combined to achieve precise control over timing

and spatial location of a manipulation (e.g., injection of a viral vector containing a gene of interest into a genetically engineered animal that ultimately results in expression of the target gene only in neurons of a specific type).

#### *Tetracycline-Inducible Systems for Modifying Gene Expression*

The tetracycline-controlled Tet-Off and Tet-On systems are used to turn gene expression on or off via the presence or absence of tetracycline or tetracycline derivatives like doxycycline (for a review see Das et al., 2016; Gossen & Bujard, 1992, 1995). The Tet-On system, for example, allows expression of a target gene in the presence of doxycycline (tetracycline is less effective in the Tet-On system). In contrast, the Tet-Off system activates expression of a gene in the absence of tetracycline or its derivatives, thus allowing gene expression to be terminated by administration of tetracycline or tetracycline-derivatives (Das et al., 2016). Thus, with both the Tet-Off and Tet-On systems, it is possible to control gene expression by administration of tetracycline or doxycycline.

Ward et al. (2012) used the Tet-Off system to control expression of the gene for the human long form of the dopamine (DA) D2 receptor (D2R) in transgenic mice containing the gene in their genome. Because they used the Tet-Off system, the human DA D2R gene was expressed in the absence and not expressed in the presence of doxycycline. Ward et al. referred to the transgenic mice expressing the human long form of the DA D2R as D2R-overexpressing mice because the mice expressed both native D2Rs and human D2Rs. Ward et al. evaluated multiple behavioral measures in control mice and D2R-overexpressing mice, but for the purpose of this discussion only one of their behavioral assessments is reported here. Ward et al. evaluated responding of mice in an "effort-related choice paradigm" (Salamone et al., 1991) in which mice responded under random ratio (RR) 5, 10, and 20 schedules of evaporated milk delivery (several sessions at each RR value) in the presence of freely available home-cage chow. In control mice and DA D2R-overexpressing mice, the average number of lever presses emitted increased as the ratio value increased, but the increases were smaller in the DA D2R-overexpressing mice. In a

separate group of DA D2R-overexpressing mice fed a diet containing doxycycline, which turned off transcription of the human DA D2R gene, the average number of responses emitted at each RR schedule value was very similar to the number of responses in the control mice.

An alternative approach to assess the role of DA D2Rs in RR responding would be to use a combined factorial/reversal design for each animal. The beauty of the tetracycline-inducible system is the ability to control gene transcription via administration of tetracycline/doxycycline in a reversible manner. Thus, one group of mice containing the transgene could be used in a combined factorial/reversal design in which RR responding was evaluated repeatedly during periods of time with the gene turned on and off. An efficient approach to evaluating the effect of transgene transcription on responding under several RR schedules of reinforcement would be to arrange a three-component multiple schedule of reinforcement composed of the three RR values. A multiple schedule is one in which two or more schedules with distinct correlated stimuli alternate within session, which allows measurement of responding across multiple schedules in the same session. Repeated reversals would increase confidence in any observed changes in RR responding (in the presence of home cage chow), during periods when the transgene was being expressed versus when it was not being expressed.

For visualization, hypothetical results producing similar average results to those obtained by Ward et al. (2012) are shown in Figure 1 for three subjects. For each hypothetical subject, responding is evaluated first in the absence of doxycycline (Gene On; data points to the left of the first vertical line) and next in the presence of doxycycline (Gene Off; data points between the first and second vertical lines), followed by repeated assessments of each (technically, a combined factorial ABAB design). An important consideration when using inducible gene expression is the duration of time required for gene expression or silencing, which might depend on the gene and target tissue. If those details are known, one approach would be to suspend experimental sessions for the necessary duration of time following addition or removal of doxycycline which, for simplicity, is the assumption

for the data presentation in Figure 1. An alternative approach would be to continue experimental sessions following addition or removal of doxycycline to directly assess changes in behavior with changes in expression levels of the regulated gene. Such an approach might be particularly useful if the duration of time required for establishing or terminating gene expression is not known.

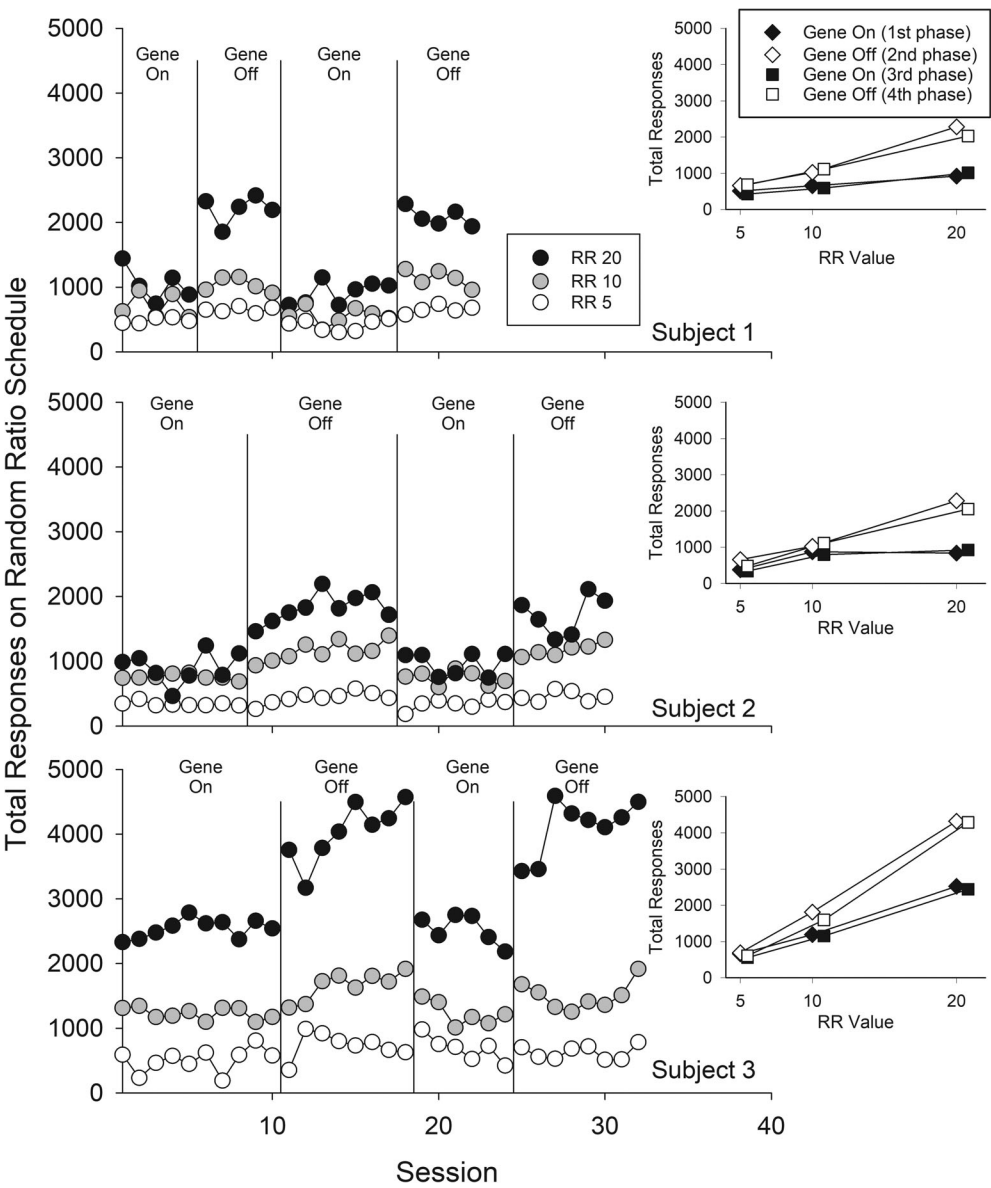
Based on the results of Ward et al. (2012), we would expect increases in responding in the larger RR schedules during the Gene Off compared to Gene On phases. Results like those shown in Figure 1 would provide compelling evidence of the causal effects of human long form DA D2R expression on RR responding reinforced by evaporated milk delivery in the presence of chow. The benefit of such an approach over the group comparison approach would be an assessment of the reliability of the effects within and across animals and a substantial reduction in the number of animals used (e.g., from four groups of seven to eight mice in the Ward et al. study to one group of mice).

### *Viral Vector Delivery of Genes*

Trifilieff et al. (2013) injected a viral vector containing the gene for the human long form of the DA D2R along with the gene for producing green fluorescence protein (GFP; a protein called a “reporter” used to visualize viral vector transfection of targeted cells) into the nucleus accumbens of mice. Trifilieff et al. reported increased responding on a progressive ratio (PR) schedule of evaporated milk reinforcement in mice overexpressing DA D2Rs in the nucleus accumbens compared to mice injected with a viral vector carrying just GFP.

In the Trifilieff et al. (2013) study, overexpression of DA D2Rs was not under reversible control. Once the target gene was introduced via viral vector injection, it was not possible to turn off transgene expression. Because the manipulation could not be reversed, a SCED investigation of the behavioral effects of introducing the gene would require the use of a multiple baseline design. For example, mice could be trained to respond on a PR schedule of evaporated milk reinforcement. Following an appropriate baseline period of responding in which stability of responding was achieved, the viral vector

**Figure 1**  
*Hypothetical Total Responses as a Function of Session, RR value, and Gene Expression Status in a Reversal Design*



*Note.* Data were generated assuming that each phase was conducted after an appropriate time following addition or removal of doxycycline to allow for gene expression or its prevention to occur. An alternative approach would be to continue sessions without any break following addition or removal of doxycycline to track the time of onset of effects from the point at which doxycycline was added or removed from the diet. Inset graphs display average responses at each RR value for the last 3 sessions of each phase (for comparison see Figure 4 of Ward et al. 2012). Data points in inset graphs are jittered to prevent overlapping of data points.

carrying the target gene could be injected at different time points in different animals to assess the effect of gene transcription (i.e., receptor expression) on behavior.

The experimental scenario is illustrated in Figure 2 using hypothetical data generated based on the PR results of Trifilieff et al. (2013). For each animal, PR responding

first is assessed prior to viral vector injection (Fig. 2, Baseline). Next, PR responding is assessed following injection of a viral vector containing the D2R transgene (and reporter) (Fig. 2, AAV-D2R). To the degree that responding changes only after injection of the viral vector in each animal and injection time points are appropriately staggered to allow comparison of baseline responding in subsequent subjects following behavioral changes in prior subjects, the behavioral changes can be reasonably attributed to the injection. As with the tetracycline-inducible systems described above, an important factor is the time required for gene expression to occur. For simplicity, the assumption made in Figure 2 is that those details are known, and experimental sessions are suspended for the necessary duration of time. An alternative would be to continue experimental sessions after an appropriate postsurgery recovery period and directly assess the onset of effects.

One concern of using a multiple baseline design to assess the effects of viral vector delivery of a transgene would be the lack of control for any possible effects of the viral vector itself. To address such possible effects, one could use another group of subjects that receive the viral vector with a reporter but without the transgene in a multiple baseline design. If the subjects receiving the viral vector containing a reporter fail to exhibit behavioral changes while those receiving a viral vector containing the transgene (and reporter) do exhibit behavioral changes, confidence that the behavioral changes in the latter group are due to the transgene would be increased. Thus, in this example, the resulting design would retain a focus on the behavior of the individual subjects while simultaneously employing group-level comparisons.

### Technologies for Controlling Neuronal Activity

Multiple technologies have been developed that allow control over neuronal activity. One technology is optogenetics, in which neurons are genetically engineered to express light-sensitive ion channels that when opened produce inhibitory or excitatory effects on the neuron (Boyden et al., 2005; for a review see Deisseroth, 2011). Another technology for

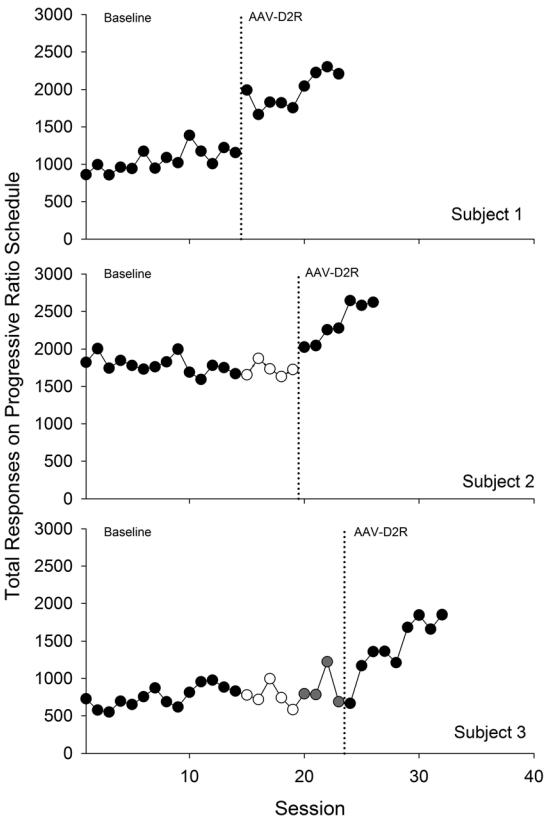
controlling neuronal activity is chemogenetics, in which neurons are genetically engineered to express specialized receptors that are only sensitive to an otherwise putatively inactive ligand. The specialized receptors and the technology are often referred to as designer receptors exclusively activated by designer drugs (DREADDs) (Armbruster et al., 2007).

#### Optogenetics

Optogenetics is a technology that utilizes genetic engineering to express light-sensitive proteins called opsins in cells (for reviews see Deisseroth, 2011; Guru et al., 2015). Once expressed in the membrane of a neuron, opsins can be stimulated by specific light wavelengths. Ion channel opsins are available that, when opened in response to light, allow influx of positively charged ions that depolarize the neuron and increase the frequency of action potentials. Ion channel opsins also are available that allow influx of negatively charged ions that, when opened in response to light, hyperpolarize the neuron and decrease the frequency of action potentials. Thus, stimulation and inhibition of neurons can be controlled by exposure to light of a specific wavelength. Light exposure is controlled by a laser fiber implanted into the target region.

Roltsch Hellard et al. (2019) utilized optogenetic stimulation to investigate the involvement of the dorsal medial striatum (DMS) in ethanol self-administration and reinstatement following extinction. For this illustration, only the effects of optogenetic stimulation on ethanol self-administration are discussed. Roltsch Hellard et al. specifically were interested in DMS neurons that projected to another area called the substantia nigra. The methods used to specifically target only DMS neurons with projections to the substantia nigra were complex and require some detailed explanation. To selectively target only those DMS neurons with projections to the substantia nigra, Roltsch Hellard et al. used two viral vector injections: one injection into the DMS and one into the substantia nigra. The first injection, into the DMS, contained the gene for halorhodopsin, an ion channel receptor that, when opened, allows influx of negatively charged chloride ions, producing hyperpolarization and decreased firing of the affected neuron. The halorhodopsin gene

**Figure 2**  
*Hypothetical Total Progressive Ratio Responses as a Function of Session and Phase in a Multiple Baseline Design*



*Note.* The dotted vertical line indicates the time at which the AAV containing the D2R gene was injected. White data points for Subject 2 and 3 indicate results obtained when Subject 1 was administered the AAV containing the D2R gene. Dark gray data points for Subject 3 indicate results obtained when Subject 2 was administered the AAV containing the D2R. For comparison see Figure 3A from Trifilieff et al. (2013).

delivered by the viral vector was Cre-dependent, which means that the gene is only expressed in the presence of an enzyme called Cre (for reviews see Mortensen, 2006; Sauer, 1998). Thus, the halorhodopsin gene was delivered to DMS neurons, but could not be expressed unless the Cre enzyme was present. The second injection, into the substantia nigra, contained the gene for the Cre enzyme. Importantly, the virus injected into the substantia nigra was a retrograde virus. A retrograde virus is one that will travel up the axons of neurons that it enters to reach the cell body of the neuron. Thus, the virus was expected to

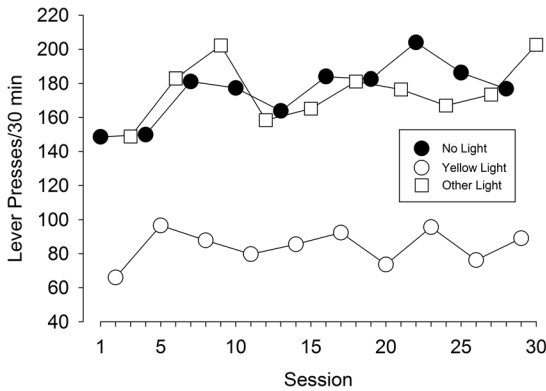
enter axon terminals in the substantia nigra, travel up the axons to reach the neuron cell bodies from which the axon terminals originated, and deliver the Cre DNA to those neuron cell bodies. Thus, by using these two injections, the Cre-dependent halorhodopsin channels should only have been expressed in the cell bodies of DMS neurons that project to the substantia nigra, because only those cell bodies would contain both Cre and Cre-dependent halorhodopsin (note that other neurons would have one or the other, but not both).

In the Roltsch Hellard et al. (2019) study, prior to viral injection, rats were trained to lever press using response-contingent delivery of 20% ethanol (v/v). Following ethanol self-administration training, rats underwent viral injection as described above and following recovery from surgery, the effects of inhibiting DMS neurons, produced by exposure to a yellow light, on ethanol self-administration were evaluated. Relative to a session occurring prior to surgery (“Baseline”) and a session (“Light Off”) 48 hr after a session during which the yellow light was on, lever-pressing reinforced by presentation of ethanol was suppressed by yellow light illumination in the DMS.

In the Roltsch Hellard et al. (2019) study, the effect of inhibiting substantia nigra-projecting DMS neurons on ethanol self-administration was assessed by comparing responding during a single baseline session, a single light on session, and a single subsequent session with the light off (48 hr post light stimulation). Although each rat was exposed to all three conditions, individual rat data were not presented nor were multiple sessions conducted with light on or off. Thus, reliability at the individual subject level cannot be assessed. However, the effects of light activation of the halorhodopsin channel receptor, a reversible manipulation, could be evaluated using a single-subject reversal design. Repeated sessions with and without light activation of the receptor would be useful to determine the reliability of the effect in individual rats. Further, the effects of a putatively “nonfunctional” light (i.e., light of a wavelength that should not activate the halorhodopsin channel receptors) also could be evaluated to control for the effects of light, per se.

Because the time precision of optogenetic stimulation is so high, a useful design to

**Figure 3**  
Hypothetical Response Rates as a Function of Session and Laser Status Using a Multielement Design



Note. For comparison see Figure 2D of Roltsch-Hellard et al. (2019).

evaluate its behavioral effects would be a multielement design in which sessions alternated between light stimulation, no light stimulation, and nonfunctional light exposure. Figure 3 illustrates some hypothetical data that might be expected, based on the Roltsch Hellard et al. (2019) results, from a multielement design in which sessions varied from day to day between Yellow Light (the wavelength that activates halorhodopsin), No Light, and Other Light manipulations in a single subject. Clear separation of rates of lever pressing would convincingly demonstrate the causal impact of light illumination of halorhodopsin-expressing neurons on ethanol self-administration. Replication of similar outcomes with additional subjects would bolster the intersubject reliability of the effect. Finally, selectivity of the effects could be examined by evaluating the effects of halorhodopsin stimulation on responding reinforced by other consequences (e.g., sucrose presentation).

### Chemogenetics

Chemogenetics is a technology that utilizes genetic engineering to produce cellular expression of receptors that can be activated by administration of an otherwise putatively inactive molecule such as clozapine-N-oxide (CNO) (for a review see Roth, 2016). A variety of receptor types have been developed based on modification of human muscarinic (G-protein coupled) receptors. When bound by CNO, the modified human muscarinic receptors produce G-protein-mediated excitatory or inhibitory effects on the receptor-expressing

neuron, depending on the specific type of receptor expressed. The receptors are often referred to as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Expression of the desired receptor in neurons can be achieved via injection of a viral vector containing the gene for the target receptor. Subsequently, CNO can be administered via standard routes (e.g., in food and via intraperitoneal injection). Interestingly, transgenic mice with Cre-dependent DREADD are emerging (e.g., Akhmedov et al., 2017; Farrell et al., 2013; Zhu et al., 2016). Cre-dependent DREADD mice contain the gene for a designer receptor but require the presence of the Cre enzyme for expression of the designer receptor. Expression of the designer receptor in target neurons can be achieved by crossing Cre-dependent DREADD mice with mice expressing Cre in the target neurons (Cre mice are readily available from several vendors), which eliminates the need for surgery to inject a viral vector containing the receptor gene.

Warthen et al. (2016) investigated the role of medial prefrontal cortex (mPFC) pyramidal neurons in mediating behavior maintained by food reinforcement using a range of behavioral procedures. Warthen et al. used viral vector injection to deliver the gene for modified human muscarinic 3 (hM3Dq) receptors (G protein-coupled excitatory receptors) into the mPFC of mice. In one portion of the study, Warthen et al. trained mice to emit nose poke responses using food reinforcement. Initially, responses in a center nose poke hole produced food pellet delivery under an FR 1 schedule of reinforcement and in subsequent sessions under FR 3 and FR 5 schedules of reinforcement. Next, mice were exposed to two sessions of a PR schedule of food delivery, with one session preceded by injection of 0.5 mg/kg CNO and the other preceded by injection of saline (order counterbalanced across mice). CNO administration increased breakpoint, total nose poke responses, and reinforcers obtained relative to saline when mice were food-restricted but not when mice were fed ad lib. Although each mouse was administered CNO and saline, single mouse data were not presented nor were multiple sessions conducted with CNO or saline administration. Thus, reliability at the individual subject level cannot be assessed. Given the

reversible nature of CNO administration, the effects of CNO and saline administration could be evaluated repeatedly to assess the reliability of the effects of CNO administration on food-reinforced PR responding. Replication of similar outcomes with additional subjects would bolster the intersubject reliability of the effect.

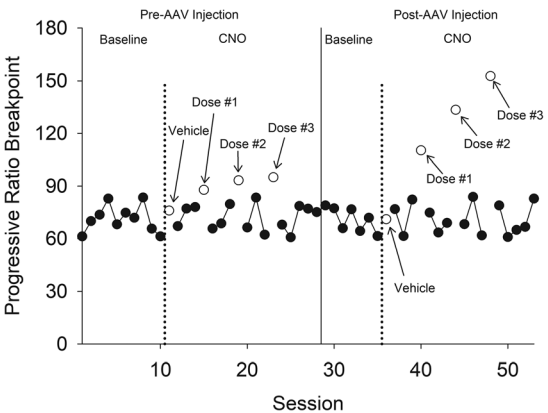
A SCED approach to evaluate the impact of CNO administration on PR responding in mice expressing hM3Dq receptors in the mPFC could be conducted using a design with reversal phases and probe sessions to assess effects of CNO and vehicle injection (a complex design that does not fit neatly into one of the described designs). First, mice could be trained to respond under the PR schedule of food reinforcement with repeated observations of the breakpoint (Pre-AAV Injection Baseline; Fig. 4). Once stability of daily breakpoint values was achieved, the effect of vehicle and different doses of CNO could be evaluated using occasional “probe” sessions (Pre-AAV injection CNO; Fig. 4). After evaluating the effects of vehicle and CNO administration on PR breakpoint, the viral vector containing the gene for hM3Dq receptors

could be injected into the mPFC, and following an appropriate surgery recovery time, PR sessions reinitiated to evaluate the potential impact of the injection of the viral vector expressing hM3Dq receptors (Post-AAV Injection Baseline; Fig. 4). Following achievement of stability in daily PR breakpoint values, a second round of probe sessions could be conducted to evaluate the impact of CNO administration on breakpoints (Post-AAV Injection CNO; Fig. 4).

The benefits of the approach detailed in Figure 4 would be the direct assessment of the reliability of effects of CNO administration within a single subject (note that behaviorally active doses of CNO could be readministered occasionally to ascertain the reliability of those effects). Importantly, as with any drug, the timing of repeated drug injections must be carefully considered in light of known pharmacokinetic and pharmacodynamic actions to avoid carryover effects from one administration to another. As described above, to the degree that carryover effects occur, they must be addressed directly in SCED research rather than avoided through the use of group designs. Further, due to concerns about back metabolism of CNO to clozapine (Gomez et al., 2017; Ilg et al., 2018; MacLaren et al., 2016; Manvich et al., 2018), effects of CNO that occurred after but not before AAV injection could be more confidently attributed to activation of the hM3Dq receptors as opposed to off-target actions of clozapine. Finally, there is continued development of ligands with promise of selective activation of designer receptors without concerns about back metabolism to an active molecule or other resulting off-target effects (Bonaventura et al., 2019).

Mahler et al. (2019) investigated the role of DA neurons in the ventral tegmental area (VTA) in behavioral economic demand for cocaine self-administration. Mahler et al. used rats expressing Cre-dependent hM3Dq (excitatory), rM3Ds (excitatory), or hM4Di (inhibitory) receptors in VTA DA neurons. Cre-dependent modified receptors were introduced via viral injection into the VTA of tyrosine hydroxylase (TH)::Cre transgenic rats (Mahler et al., 2014; Witten et al., 2011), which express the Cre enzyme only in TH-positive neurons. As a result, the designer receptors were selectively expressed in TH-positive neurons of the VTA. Rats were trained

**Figure 4**  
*Hypothetical Progressive Ratio Breakpoints as a Function of Session and Phase Using a Complex Design to Assess the Effects of CNO Prior to and After Administration of a DREADD-Containing Viral Vector*



*Note.* Dose numbers indicate unspecified values of increasing doses. A small effect of CNO in the absence of DREADD expression was assumed to demonstrate the potential benefit of the design for assessing the effect of CNO in the absence and presence of DREADD expression in the same animal. The dotted vertical lines indicate the points in time at which daily breakpoint values were deemed stable enough for probe sessions to occur. The solid vertical line indicates the point in time when the viral vector containing the hM3Dq receptor gene was injected.

to lever press using 0.2 mg/inf cocaine as a reinforcer and subsequently underwent several behavioral evaluations. The focus here will be on the portion of the study that assessed the effects of CNO administration on demand for cocaine. During cocaine demand sessions, the schedule of cocaine reinforcement was FR 1 and the available dose of cocaine decreased every 10 min from 358.4 – 1.1 microg/inj (see Bentzley et al., 2013 for detailed methods). Hursh and Silberberg's (2008) exponential model of demand was fitted to consumption of cocaine in mg in each 10 min bin as a function of price (responses/mg) in each bin. In hM3Dq-expressing rats, CNO dose-dependently decreased the parameter  $Q_0$ , which represents demand at zero price (i.e., maximal consumption), and  $\alpha$ , which is inversely related to reinforcer effectiveness. In hM4Di-expressing rats, CNO dose-dependently increased  $Q_0$  and  $\alpha$ . In the rM3Ds-expressing rats, CNO did not substantially alter either parameter. CNO and vehicle were administered and demand curves obtained in each rat, but data were not presented to allow assessment of reliability within or across individuals. Although the behavioral economic demand approach adds more complexity, because CNO is eliminated from the body, its effects are suitable for evaluation using a SCED.

A SCED approach to evaluate the effect of hM3Dq and hM4Di receptor stimulation on demand for cocaine could be conducted as

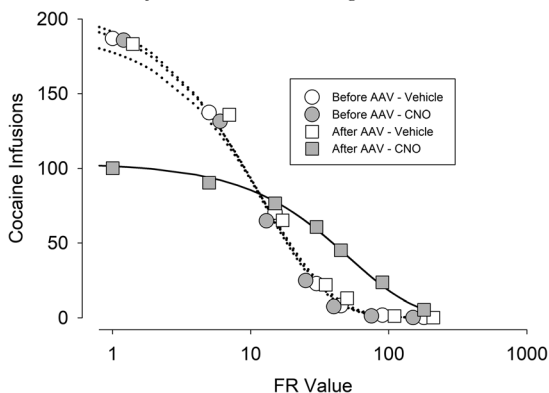
follows. Following cocaine self-administration training, a demand curve for cocaine following vehicle injection could be obtained using the procedure of Bentzley et al. (2013) (Fig. 5, "Before AAV - Vehicle"). Next, the effects of CNO on cocaine demand could be evaluated (Fig. 5, "Before AAV - CNO"). Following injection of the viral vector containing the gene for the target DREADD could be administered and cocaine demand following vehicle injection later reassessed (Fig. 5, "After AAV - Vehicle"). Finally, CNO could be administered pre-session and cocaine demand assessed (Fig. 5, "After AAV - CNO"). Although the effects of only a single CNO dose are shown in Figure 5, a range of doses could be administered to rigorously determine the effects of neuronal stimulation and inhibition via DREADD activation on cocaine demand.

### Future Directions and Final Thoughts

This paper argues that technologies currently available that allow initiation and termination of biological processes in the brain permit the application of SCED methods to the study of the relation between such processes and behavior. Application of SCED methods to study brain-behavior relations will deepen our understanding of the causes of behavior and likely will lead to improved therapeutic interventions in domains involving behavior (cf. Thompson, 2007). Such studies should be conducted via collaborations between behavior analysts and behavioral neuroscientists, bringing together their respective areas of expertise. In addition to the scientific value of increasing our understanding of the causes of behavior, there are practical benefits for behavior analysts and behavioral neuroscientists. For behavior analysts, the benefits include increased opportunities for interdisciplinary research efforts, increased outlets for publication, and increased opportunities for obtaining research funding. For behavioral neuroscientists, the benefits include facilitation of internal validity and increased reproducibility of findings stemming from the built-in replications inherent in SCED methodology, generation of findings that apply to individual organisms, and decreased costs and labor stemming from a decrease in the number of animals required.

There are a variety of directions for investigation utilizing the technologies

**Figure 5**  
*Hypothetical Demand Curves for Cocaine from Each of Four Conditions Across Which CNO Was Administered Before and After Administration of a DREADD-Containing Viral Vector*



*Note.* For comparison, see Figure 5A and 5B of Mahler et al. (2019). Data points are jittered to prevent overlap.



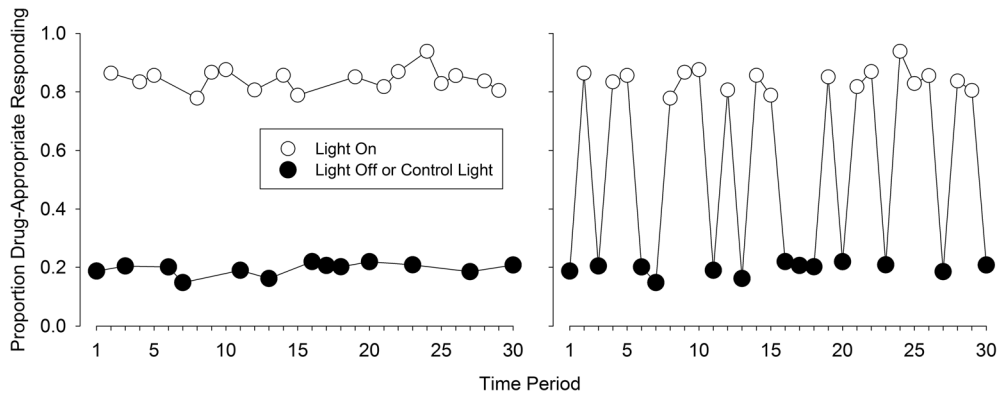
described in this paper that could be of interest to behavioral researchers. Control over gene transcription in a regionally, temporally, and neuron-type specific manner allows investigation of questions such as the role of neurotransmitter receptors in mediating environment-behavior relations with a precision not previously possible using gene knockout animals. For example, future research could determine, in a neuron type- and region-specific manner, which of the various DA receptor subtypes are necessary for the reinforcing (i.e., “abuse-related”) effects of dopaminergic drugs such as cocaine and amphetamine. Similar work could be done to determine which of the various DA receptor subtypes and what regions of their expression contribute to the reinforcing effects of “natural” reinforcers such as food and water (see Soto et al., 2011; Soto et al., 2016 for previous attempts to evaluate this question in knockout mice).

As discussed by Thompson (2007), biological processes and events (endogenous variables in Thompson’s terminology) may function as motivating operations and as stimuli with discriminative and reinforcing consequences. Control over neuronal activity in a regionally, temporally, and neuron-type specific manner can determine the role of biological processes and events with functions outlined by Thompson. For example, given the reasonable assumption that the subjective effects of drugs arise from drug actions at

target sites in the brain, one could use optogenetic or DREADD technologies to probe the neurobiological systems that generate the subjective effects of drugs. Optogenetic or DREADD-based stimulation or inhibition of specific target neurons could be utilized in animals trained to discriminate a drug from vehicle to determine whether modulation of the target neurons “substitutes” for the subjective effects of a drug (i.e., functions as a discriminative stimulus for drug-appropriate responding). Given the temporal precision of optogenetic stimulation, one could conceivably demonstrate control over responding on a within-session basis at the level of individual animals (see Fig. 6). Modulation of the subjective effects of a drug through DREADD-based stimulation has been demonstrated (e.g., Jaramillo et al., 2018), but to my knowledge, whether such stimulation can substitute for a drug’s subjective effects remains unexplored.

Another exciting possibility would be to determine whether optogenetic- or DREADD-based modulation of targeted neuronal activity could itself be trained as a discriminative stimulus. If so, results such as those shown in Figure 6 might be obtained by providing reinforcement for responding on one alternative in the presence of light stimulation and by providing reinforcement for responding on another alternative in the absence of light stimulation or in the presence of another light frequency (substituting “Light On-Appropriate Responding” for “Drug-Appropriate Responding”). The discriminative-

**Figure 6**  
*Hypothetical Proportion of Responses on a Drug-Appropriate Alternative as a Function of Time Period and the Status of an Optogenetic Laser*



*Note.* The graph on the left displays hypothetical data using a conventional graphing style and the graph on the right displays the data using a graphing style designed to highlight the precise temporal control that could be observed.

stimulus effects of DREADD-based modulation of neuronal activity could be investigated similarly although on a session-by-session rather than a within-session basis due to the duration of action of CNO.

The possibility that optogenetic or DREADD-based modulation of neuronal activity could function as a discriminative stimulus appears likely based on research on fear memories. In two groundbreaking studies, researchers demonstrated that optogenetic stimulation of neurons can itself elicit a response conditioned in a specific environment or be used to generate a conditioned response to a specific environment. In the first study, Liu et al. (2012) demonstrated that neurons previously activated in a shock-paired environment elicited freezing when those neurons were stimulated, via optogenetics, in a different environment. In a second study, the same group demonstrated that an environment never paired with shock elicited freezing if subsequent optogenetic stimulation of the neurons that were active during initial exposure to the environment was paired with shock in a different environment (Ramirez et al., 2013). Although discussed in terms of memory, this research also raises fascinating questions about the possible stimulus functions of such stimulation, such as whether conditioned responses would undergo extinction with repeated stimulation and exhibit other classical conditioning phenomena. Such research demonstrates that stimulation of specific populations of neurons can function as conditioned stimuli and strongly suggests that such stimulation could exert discriminative control over responding.

Further lines of research involve evaluating neuronal excitation or inhibition as consequences of behavior. It has long been known that stimulation of certain populations of neurons can reinforce behavior that leads to such stimulation (Olds & Milner, 1954). Similarly, the reinforcing consequences of optogenetic stimulation of specific neuronal populations have been demonstrated (e.g., Steidl & Veverka, 2015; Stuber et al., 2011; Witten et al., 2011). To my knowledge, the punishing consequences of optogenetic- and DREADD-based modulation of neuronal activity on reinforced responding and the negative reinforcing effects of such stimulation remain unexplored (although see Tan et al., 2012, for

an example of using optogenetic stimulation to produce conditioned place aversion). Finally, whether direct modulation of specific neuronal populations can establish or abolish certain events as reinforcers or punishers (i.e., function as motivating operations) represents another interesting line of possible research.

The technologies described here represent opportunities for merging the rapidly advancing field of neuroscience with the methods of behavior analysis, methods proven useful for the analysis of the environmental determinants of behavior. These technologies by no means represent an exhaustive coverage of those technologies utilized in behavioral neuroscience that could fruitfully be applied using SCEDs. Notably, technologies offering control with less invasiveness are being developed (e.g., “fiberless” optogenetics; Miyazaki et al., 2019). Behavior analysts and behavioral neuroscientists should collaborate on efforts to study the neurobiological determinants of behavior in individual organisms. Collaborations between behavior analysts and behavioral neuroscientists will bring the world within the skin under experimental control and create a thoroughgoing analysis of the determinants of behavior.

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